

ABSTRACT

TITLE: Evaluation of effects of various temperatures on potency of premixed isophane insulin in experimental animal model.

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BACKGROUND:

Insulin being proteinaceous in nature can undergo physical or chemical degradation due to various factors. Potency of insulin refers to the efficacy of insulin in reducing the blood glucose levels. It is affected by multiple factors such as exposure to light, vibration, variation in storage temperatures and duration of storage. Inappropriate storage of insulin can also decrease insulin's potency, which could contribute to poor response to insulin therapy.

OBJECTIVES

1) To determine the safe dose of human insulin mixtard in male Wistar rats which can be administered to rats without inducing hypoglycemia, by doing a validation study and also to determine the peak time of action which is essential to decide the appropriate time points for blood specimen collection in rats.

2) To evaluate the impact of different temperatures (5°C, 25°C and 40°C) and duration of storage on the potency of human insulin mixtard when stored over a period of 35 days using an experimental Wistar rat model.

METHODS:

The optimal dose of insulin (0.25U/kg body weight) and time points of blood sampling (pre dose, 0.5, 1, 1.5 and 4, 6, 8 hours) were selected for the main study based on the results from a validation study conducted in our laboratory. Eighteen male Wistar rats of 20-22 weeks of age and weighing 250-300g were equally divided into 3 temperature groups- 5°C group, 25°C group, and 40°C group. Each group served as its own control and received normal saline at specific temperatures as the placebo. After overnight fasting, insulin tolerance test was done by intraperitoneal injection of insulin (test) or saline (placebo) stored at the 3 different temperatures i.e. 5°C, 25°C, and 40°C. The blood sampling was done by cutting the tip of the rat tail using a scalpel and the glucose levels are measured by glucometer at various time points as determined by the validation study i.e. at 0 hour (predose), 0.5 hour, 1 hour, 1.5 hours and 4 hours, 6 hours and 8 hours after administration of insulin. This was repeated on 1st, 7th, 21st and 35th day. The results were analyzed using R programme for statistical computing. The statistical tests used were ANOVA with post-hoc analysis and repeated measures ANOVA.

RESULTS:

1. On days 1, 7 and 35, there was a significant difference in potency with respect to the short acting part of insulin between the 3 temperature groups. (p values – 0.041, 0.018, 0.01 respectively) by ANOVA and Tukey's HSD test.

a) On day 1, potency of insulin stored at 40°C was significantly different from potency of insulin stored at 25°C (p value – 0.036).

b) On day 7, potency of insulin stored at 40°C was significantly different from potency of insulin stored at 5°C and 25°C (p values – 0.051 and 0.023 respectively).

c) On day 35, potency of insulin stored at 40°C was significantly different from potency of insulin stored at 5°C (p value – 0.008).

2. There was no difference in potency of insulin (both short acting and long acting part) in any temperature group over the study period of 35 days by repeated measures ANOVA.

CONCLUSION:

Even one day of storage of insulin stored at 40°C significantly affected its potency and biological activity, whereas insulin stored between 5°C and 25°C retained its potency as long as for 5 weeks. The reduction in the potency of insulin at higher temperatures could be one factor contributing to an apparent insulin resistance seen in rural areas during summers. The current study emphasize that diabetic patients should be properly educated about the appropriate methods of storing insulin with respect to temperature and duration of storage in order to maintain good glycemic control.

KEY WORDS: Premixed isophane insulin, temperature, potency, glucose